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surface-tethered vesicles with up to 3-4 consecutive injections per reactor. Development of ultra-small-volume fluidic platforms will enable novel ways to implement simultaneous screening of biochemical properties, molecular function or confined reactions over millions of samples while consuming total reagent volumes of few picoliters.

References:
(1) Christensen and Stamou,Soft Matter, 2007.
(2) Christensen et al.,Nature Nanotechnology, 2011.

3053-Plat
Single Molecule Fluorescence Imaging at Micromolar Concentrations
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The conventional total internal reflection (TIR) microscopy or confocal microscopy renders a detection volume of 20-200 attoliters, limiting single molecule experiments to nanomolar concentrations of fluorescently labeled reagents. However, many biological processes require higher concentrations of proteins or substrates. Here, we present a novel combination of existing techniques with confocal microscope to reduce the detection volume to below 300 zeptoliter, which represents a three orders of magnitude reduction compared to the confocal case. The sub-diffraction focal spot of stimulated emission depletion microscopy accounts for $>$ 20-fold reduction of volume in lateral direction and a simple convex lens provides $>$ 40-fold confinement in axial direction. This method should allow single molecule studies of complex processes that require transient interactions between multiple components.

Platform: Protein-Nucleic Acid Interactions

3054-Plat
Structural Basis of RNA Recognition and Activation by Innate Immune Receptor RIG-I
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RIG-I-like receptors (RLRs) of the innate immune system are the cell's principal detector of RNA viruses. These proteins distinguish between cellular and viral RNAs by recognition of Pathogen Associated Molecular Pattern (PAMP) motifs that are associated with viral RNAs. RIG-I (Retinoic acid Inducible Gene - I) is a cytosolic pathogen recognition receptor that recognizes viral RNA motifs and triggers an immune signaling cascade resulting in type-I interferon induction. RIG-I consists of three domains; the N-terminal CAspase Recruitment Domains (CARD), the central helicase domain and the C-terminal repressor domain (RD). The helicase and RD of RIG-I recognize double-stranded (ds) RNA and 5′-triphosphate RNA as foreign and activate the RIG-I CARD for signaling. However, the nature of RIG-I:RNA interaction remains unclear. To understand how the RIG-I helicase binds RNA and leads to activation, we have determined the structure of the human RIG-I helicase-RD domain bound to dsRNA and ADP•BeF3. The structure of the ternary complex reveals a major contribution of the helicase to RNA binding and a synergy between the helicase and RD in the recognition of blunt-ended dsRNA. Helicase-RD organizes into a ring with the helicase utilizing previously uncharacterized motifs to specifically recognize dsRNA. Additional biophysical and biochemical results demonstrate that RIG-I, in absence of RNA is flexible and becomes more compact upon RNA binding. These results provide a greater understanding of the cellular response and immune activation to viral infection. However, the role of ATPase/helicase function of RIG-I remains elusive. The RIG-I helicase-RD represents the first structure of an RNA helicase bound to dsRNA and provides a new perspective in understanding how other homologous RNA helicases may engage their targets.

3055-Plat
Oligomerization of HIV-1 Restriction Factor APOBEC3G Transforms it from a Fast Enzyme to a Slow Nucleic Acid Binding Protein
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Human APOBEC3G (A3G) is a cellular protein that inhibits reverse transcription and replication of human immunodeficiency virus type-1 (HIV-1) in the absence of the viral protein Vif. A3G impairs viral replication by two different mechanisms, which both rely on its ability to bind single-stranded nucleic acids. First, A3G deaminates cytidine bases of viral single-stranded DNA (ssDNA). Secondly, A3G blocks DNA synthesis by reverse transcriptase (RT), the viral DNA polymerase, by a mechanism independent of catalytic activity. Seven A3G proteins are packaged per HIV-1 virion, requiring that each molecule rapidly locate deamination sites on viral ssDNA, which is a transient intermediate during reverse transcription. In contrast, the roadblock mechanism, a model in which A3G oligomerizes on the viral template strand and blocks RT-catalyzed DNA elongation, requires an extremely slow off-rate from single-stranded nucleic acids. We hypothesize that A3G exhibits fast binding kinetics as a dimer, enabling rapid deamination activity, and slow kinetics as an oligomer, preventing RT from elongating viral DNA. We use optical tweezers, in combination with fluorescence anisotropy and surface plasmon resonance, to quantify both types of binding kinetics. DNA stretching experiments reveal that the time constant for oligomerization, ranging from 200 to 1000 s, is inversely dependent on protein concentration. The apparent dissociation constant of A3G oligomerization decreases exponentially with ssDNA incubation time, dropping by an order of magnitude in 1000 s, which suggests that fast binding of catalytically active dimers converts to oligomerization on this timescale. This slow association and dissociation of A3G oligomers, which is consistent with ensemble methods, supports the roadblock hypothesis. Collectively, our measurements quantitatively characterize the complex, highly unusual nucleic acid binding kinetics of A3G responsible for its dual mechanism for inhibiting viral replication.

3056-Plat
Trbp Sliding Promotes Dicer's Cleavage Activity
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Transactivation response (TAR) RNA binding protein (TRBP) is an essential cofactor of Dicer in the RNA interference pathway. TRBP enhances Dicer's processing of both microRNA (miRNA) and small interfering RNA (siRNA) precursors, yet the mechanistic basis of this effect has not been elucidated. Here we report a robust sliding activity of TRBP exclusively on double stranded RNA (dsRNA). TRBP exhibits ATP-independent motion selectively on double stranded RNA in a length dependent manner. We find that the first two double stranded RNA binding domains (dsRBDs) of TRBP, which stimulate the dicing activity, are also directly responsible for the sliding motion, whereas the third dsRBD is dispensable. The same sliding motion was also observed in two other dsRBD-possessing proteins, PACT, and R3D1, implying a universality of sliding activity in this family of proteins. When in complex, Dicer-TRBP displayed two modes of binding to dsRNA: static interaction and dynamic sliding. Upon stimulation of RNA cleavage, the sliding molecules, not the static molecules, disappeared selectively, strongly suggesting that the sliding motion of Dicer-TRBP promotes dicing activity. Our study demonstrates a novel mode of motion, sliding on dsRNA exhibited by dsRBD containing proteins, and suggests that such activity of TRBP leads to an enhanced catalytic cleavage of Dicer.

3057-Plat
Automated DNA Tracing on AFM Images Helps the Study of 186 Repressor-DNA Interactions
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AFM images of DNA deposited on mica surface were recorded and single molecules were traced using an automated program developed with the improved thinning algorithm of Brugal and Chassery. This program dramatically improves throughput such that thousands of DNA traces may be obtained within a day. The program was used to analyze AFM images of DNA-186 repressor nucleoprotein complexes relevant to the regulation of the 186 bacteriophage genetic switch. The location of protein particles on the DNA, their size and volume could be generated automatically. The result showed that the 186 bacteriophage repressor, 186 CI, aggregates into a disc-shaped heptamer with the help of DNA and explains why this oligomerization state of the repressor had not been observed in sedimentation equilibrium experiments performed on protein solutions. Furthermore, the high statistics achievable with this program allowed